Semiannual Progress Report NASA Grant NsG-692
September 1, 1967 through February 29, 1968
Project Title: Mass Spectrometric Elucidation of Volatilizable Dilute Constituents in Tissue Preparations, in vitro.
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Prosecution of this project to date has centered on devising an analytical system capable of detecting $^{14}N/^{15}N$ ratios present in nitrogen-containing intermediates elaborated by mammalian cell preparations[1] in the presence of ^{15}N-labelled substrate.

The analytical system consists of a Time-of-Flight mass spectrometer connected in tandem with a gas chromatograph by means of porous, fritted glass effusion chamber. The ^{15}N-containing sample is injected into a coiled, 2 feet long, 1/8" O.D. stainless steel column packed with a Silyl-8 treated porous polymer (Porapak R) where an initial separation of H_2O (M/e 18) and $^{15}NH_3$ (M/e 18) takes place, prior to introduction of the gas stream into the mass spectrometer.

This system provides the means for determining ratios of ions in the range of M/e 14-18 (S.D.>0.05) produced from a mixture of ^{14}N and ^{15}N ammonia. This degree of accuracy should be sufficient for accurate measurement of incorporation of ^{15}N into metabolic intermediates.

The specific reactions being studied are those involved in asparagine biosynthesis by Clone 929 mouse cells[1]. These may be summarized as follows:

[1]Clone 929 mouse cells grown in a completely defined medium by Dr. Paul A. Kitos, Department of Comparative Biochemistry and Physiology, University of Kansas, Lawrence, Kansas.

(a) $H_2{}^*NC(CH_2)_2CH(NH_2)COH + HOCCH_2CH(NH_2)C{-}OH \xrightarrow{ATP}$

$HOC(CH_2)_2CH(NH_2)COH + H_2{}^*NCCH_2CH(NH_2)COH$

or:

(b) $HOCCH_2CH(NH_2)C{-}OH + {}^*NH_3 \xrightarrow{ATP} H_2{}^*NCCH_2CH(NH_2)COH$

*designates ^{15}N.

Preliminary experiments using Clone 929 mouse cells indicate that this system synthesizes asparagine from aspartic acid utilizing glutamine as the preferred nitrogen source and requires ATP.

Reasons for selecting this reaction are as follows:

1) A well defined cell and growth medium system is available.
2) $^{14}N/^{15}N$ ratio determinations can be made with a low resolution mass spectrometer.
3) Some insight into the ATP requirement may be possible.
4) Asparagine metabolism has been implicated in certain meoplasms, including leukemia.

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